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Evaluation of the extent of urinary excretion of 19-norandrosterone in women and considerations regarding norethisterone administration

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Introduction

Our objective was to provide a solid scientific foundation on which to justify a decision that may lead to disciplinary action following an adverse finding of 19-norandrosterone, including cases that are associated with the use of norethisterone. To achieve the objective, we evaluated: (i) the excretion of 19-norandrosterone by a large number of healthy women, including those using contraceptives containing norethisterone; (ii) the purity of pharmaceutical preparations of norethisterone with respect to the potential impurity 19-norandrostenedione; (iii) the contribution of administered norethisterone preparations to the production of urinary 19-norandrosterone.

Results and Discussion

Single urine specimens were collected from female volunteers (n = 1202), of whom 38 were taking norethisterone-containing contraceptives. Taking specific gravity into account, only one sample exceeded the 19-norandrosterone reporting threshold, having a 19-norandrosterone concentration of 4.1 ng/mL (S.G. = 1.025; adjusted reporting threshold 2.5 ng/mL). This sample was from a user of a norethisterone-containing contraceptive. The highest concentration observed in women not using norethisterone-containing contraceptives was 1.7 ng/mL (S.G. = 1.026, adjusted reporting threshold 2.6 ng/mL).

Of special interest were samples close to the adjusted reporting threshold (i.e. greater than 1 ng/mL) from women not using norethisterone. These samples all had an S.G > 1.020 and were analytically challenging because of a very complex sample matrix in which the presence of components in large concentration caused disruption of the chromatography and column overload. These, therefore, were further analysed using a different approach. In this different method [2,2,4,4-$^{2}H_4$]-19-norandrosterone glucuronide was added as an internal standard to three 1 mL aliquots of each urine sample, such that the aglycone was at a concentration equivalent to the adjusted reporting threshold. The derivatised sample extract was analysed using the same chromatographic conditions but selected ion monitoring on a GC-MSD. Using this different approach none of these samples exceeded the reporting threshold, as judged by comparison of peak heights (m/z 405 v m/z 409) but two samples were virtually identical to the threshold.

In the chemical synthesis of norethisterone, the intermediate 19-nortestosterone (nandrolone) is efficiently converted to 19-norandrostenedione. The European Pharmacopoeia states that the amount of 19-norandrostenedione should be not more than 0.1 % of the norethisterone. An LC-MS/MS assay we developed for pharmaceutical analysis of 19-norandrostenedione showed that norethisterone formulations contained up to 1 µg per 5 mg tablet (0.02 % w/w) and in oral contraceptives up to 0.5 µg per tablet (0.05 % w/w).

A crossover study was performed with 10 female volunteers where norethisterone was administered either as chromatographically purified material (<2 ng 19-norandrostenedione per 5mg of norethisterone) or as 5 mg tablets containing 1 µg of 19-norandrostenedione/tablet. The regimen was 3 x 5 mg per day for 10 days, which resulted in a maximum urinary 19-norandrosterone concentration of 51 ng/mL with the purified material and 63 ng/mL with the tablet. The concentration ratio of 19-norandrosterone to tetrahydronorethisterone (3α,5β-THN) was 0.09 in both cases. As a follow-up, a single 1 µg dose of 19-norandrostenedione was administered, resulting in a maximum urinary concentration of 2.4 ng/mL of 19-norandrosterone 2 h post-administration. Finally, administration to women (n = 30) of a single dose of an oral contraceptive containing 1 mg of norethisterone and 0.5 µg of 19-norandrostenedione resulted in a urinary 19-norandrosterone concentration of 9.1 ng/mL up to 4 h post-administration, with a maximum 19-norandrosterone/5β-THN ratio of 0.36.

The data from three case studies showed that they are all within the spread of values from the administration studies (Figure 1). Data from an earlier study where urine collected from women was found to contain 19-NA > 2ng/mL and 3α,5β-THN were found to be within the spread of values from the oral contraceptive study (Figure 1). From the data set generated in this study we can confidently conclude that these findings are consistent with norethisterone administration alone rather than co-administration of nandrolone or its prohormones. Such an approach is very useful to laboratories, experts and sports disciplinary panels, in that it avoids the necessity of time-consuming follow-up investigations and should be helpful to those athletes involved for the speedy resolution as to whether their sample is negative or not.

Our findings support: (i) that the revised reporting threshold for 19-norandrosterone for women is acceptable; (ii) the contribution of the tablet impurity 19-norandrostenedione to urinary 19-norandrosterone is very small in comparison to that from metabolism of norethisterone; (iii) the presence of tetrahydronorethisterone isomers in urine may not constitute a valid defence of an adverse finding for 19-norandrosterone where the reporting threshold is significantly exceeded in combination with the 19-norandrosterone/3α,5β-THN ratio being greatly augmented.

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References

Full details have been accepted for publication elsewhere:
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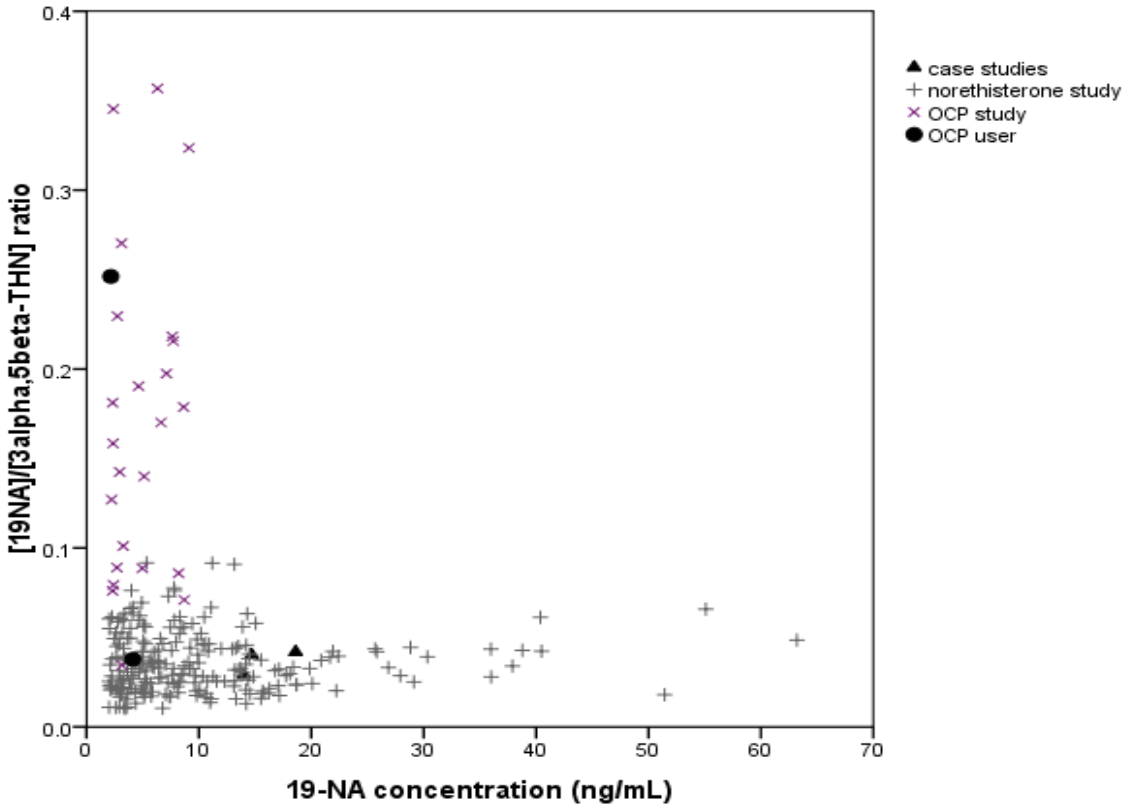

Figure 1. 19-NA concentration versus the concentration ratio of 19-NA to 3α,5β-THN for therapeutic norethisterone administration urines (+), oral contraceptive administration urines (x), norethisterone contraceptive users (●) and case studies of women on norethisterone treatment where the 19-NA concentration was greater than 2 ng/mL and 3α,5β-THN was detected (▲).